Chapter 8
Fear Conditioning and Extinction

Bronwyn M. Graham and Mohammed R. Milad

List of Abbreviations

CS	Conditioned stimulus, an initially neutral stimulus that is paired with an aversive stimulus during fear conditioning;
US	Unconditioned stimulus, an innately aversive stimulus that is paired with the to-be-conditioned stimulus during fear conditioning;
CR	Conditioned response, a species-specific defensive reaction induced by the non-reinforced presentation of a conditioned stimulus;
SCR	Skin conductance response, a psychophysiological index of arousal in humans;
BLA	Basolateral nucleus of the amygdala;
CEA	Central nucleus of the amygdala;
DH	Dorsal hippocampus;
vmPFC	Ventromedial prefrontal cortex;
PL	Prelimbic division of the ventromedial prefrontal cortex;
IL	Infralimbic division of the ventromedial prefrontal cortex;
BDNF	Brain-derived neurotrophic factor;
dAC	Dorsal anterior cingulate;
PTSD	Post-traumatic stress disorder, an anxiety disorder that affects 15–20% of people exposed to a traumatic event;
EMG	Electromyography, the measure of electrical activity produced by skeletal muscles; an index of startle in humans.

B.M. Graham (✉)
School of Psychology, University of New South Wales, Sydney, Australia
e-mail: bgraham@psy.unsw.edu.au

M.R. Milad
Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, USA
e-mail: milad@nmr.mgh.harvard.edu

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8.1 Fear Conditioning: Definition and Overview

8.1.1 Acquisition of Fear: Cued and Contextual Fear Conditioning

Pavlovian fear conditioning is a commonly used laboratory procedure in both non-human animals and humans (Milad et al. 2006). Typically in this procedure, an initially neutral to-be-conditioned stimulus (CS; e.g., a light or a tone) is paired with an aversive unconditioned stimulus (US; e.g., a mild electrical shock). After several pairings, the subject starts to exhibit conditioned fear responses (CRs) to presentations of the CS itself, having learnt that the CS predicts the US. This type of fear conditioning is known as "cued conditioning"; however, fear conditioning can also occur to distinct environments. This type of conditioning, known as "contextual fear conditioning," involves the presentation of an unsignaled US in a specific context. Fear conditioning is a robust phenomenon in the laboratory in human subjects (Hofmann et al. 2010), who can be conditioned to fear ecologically relevant stimuli (such as images of negative or fearful faces, and innately fear-provoking animals) or completely neutral stimuli (e.g., shapes, neutral images).

Fear conditioning produces both overlapping and species-specific fear responses in non-human animals and humans. In rodents, conditioned freezing (defined as the absence of all movements except that used for respiration; Fanselow 1980), fear-potentiated startle responses (defined as an increase in the reflexive startle response that occurs in the presence of the CS versus a neutral stimulus; Davis 1990), and conditioned suppression of feeding (where food intake decreases in the presence of the CS; Bodnoff et al. 1988) are among the most commonly used measures of fear (see Cryan and Holmes 2005, for review). In humans, skin-conductance responses (SCRs) and fear-potentiated startle responses are the most commonly used psychophysiological measures of fear (Milad et al. 2006). Fear conditioning has been viewed as a valid model of the symptoms of anxiety, as it induces similar fear responses to those seen in humans with anxiety disorders (Cryan and Holmes 2005).

8.1.2 Inhibition of Fear: Extinction

Once conditioned, fear responses to both a conditioned context and a discrete CS can be reduced via a procedure known as extinction training. During such training, the subject is repeatedly exposed to the feared CS in the absence of any reinforcement. After several presentations, fear responses gradually decline as the subject learns that the stimulus no longer predicts the aversive outcome. The diminution of fear responses during extinction training is known as "within-session extinction training," or "extinction learning" (Myers and Davis 2007). At a later time point (usually the next day), subjects can also be tested for long-term maintenance

of extinction, known as "extinction recall" or "extinction retention" (Graham and Milad 2011). Good extinction recall is indexed by low levels of conditioned responding, whereas poor extinction recall is indexed by recovered conditioned fear responses. Exposure therapy, which is a commonly used and empirically validated treatment for anxiety disorders, is based on the extinction procedure (Foa 2011; Wolpe 1954). During exposure therapy, the individual is exposed to fear-eliciting cues, situations, and outcomes, in the absence of any danger, which challenges unrealistic cognitions about the probability and actual cost of negative events, and ultimately results in a reduction in anxiety (Otto et al. 2004).

8.2 Cognitive and Neural Mechanisms of Fear Conditioning and Extinction

8.2.1 Cognitive and Behavioral Theories of Conditioning and Extinction

In addition to its utility in modeling the symptoms of anxiety, fear conditioning is just as often used as a task with which to examine the cognitive, behavioral, and neurobiological mechanisms behind memory formation. Cued fear conditioning is most commonly conceptualized as involving the formation of an associative memory, dependent on an understanding of the temporal relationship between the CS and US (Maren 2001). Contextual fear conditioning also relies on the formation of an association between the context and the US; however, it is different from cued conditioning in the sense that a context does not provide temporal information regarding the onset of the US and requires the integration of information from multiple senses (e.g., hearing, sight, smell) to form a contextual representation (Maren et al. 1998; Rudy et al. 2004). In this way, cued and contextual fear conditioning rely on somewhat different neurobiological mechanisms (see below).

Although extinction causes reductions in conditioned responding, it is a process distinct from forgetting as it depends on the animal being presented the non-reinforced cue. If the animal receives no such presentations, its fear for the cue will remain across weeks, and even years (Gale et al. 2004). Fear extinction was originally thought to reflect unlearning of the fear conditioning memory (Rescorla and Wagner 1972). However, several lines of evidence have led to the now commonly accepted view that, like fear conditioning, fear extinction also appears to depend on the formation of a new extinction memory that coexists with the original fear memory (reviewed in Myers and Davis 2007; Quirk and Mueller 2008). The main evidence that the fear conditioning memory still exists following extinction is that several manipulations have been shown to lead to recovery of fear responses. For example, fear responses often recover when the subject presented an extinguished cue in a different context to that in which extinction training took place, a phenomenon known as "renewal" (Bouton and Bolles 1979a). In addition,

fear responses often recover following the occurrence of a mildly stressful event, such as an unsignaled footshock, known as "reinstatement" (Bouton and Bolles 1979b). Finally, fear responses also have been shown to recover with increasing intervals between extinction training and test, known as "spontaneous recovery" (Bouton 1993). Together, these findings have prompted the theory that extinction involves the formation of a new memory that is contextually gated (Bouton 2002). According to this account, during extinction, the subject learns that the CS no longer predicts the US in that specific context. Therefore, when the cue is presented in a context other than that in which it was extinguished, fear of the cue returns. The change in the context that precipitates relapse may be the physical environment in which the extinguished cue is presented, as occurs in renewal, or it may reflect changes in the temporal context as occurs in spontaneous recovery (Bouton 1993). Finally, it may reflect changes in the affective value of the extinction context itself, as in the case of reinstatement, where an unsignaled stressor only elicits recovered fear responses when it occurs in the same context as extinction (Bouton and Bolles 1979b).

Although it is well accepted that fear extinction at least partly occurs via new learning, more recently some researchers have proposed that extinction may also lead to partial erasure of the original fear conditioning memory. For one thing, relapse following fear extinction is rarely complete—subsequent to renewal, reinstatement, and spontaneous recovery procedures, subjects typically express a level of fear that is less than that expressed following fear conditioning (Delamater 2004). For another, the mechanisms underlying fear extinction appear to change across development. In contrast to adult rodents, young rodents exhibit a relapse-resistant form of extinction that does not depend on the same neural or molecular substrates of extinction that occurs during adulthood, and some have suggested that extinction during early development involves fear erasure (Gogolla et al. 2009; Kim and Richardson 2010). It is therefore possible that extinction in adulthood retains some of the qualities of extinction during development, but that the relative contribution of the mechanisms switches such that new learning is now dominant. Indeed, neurobiological evidence supports the notion that extinction during adulthood reverses some of the changes caused by fear conditioning, in line with an erasure hypothesis (see below). These findings have led some researchers to propose "hybrid" models of extinction, which purport that extinction results from multiple mechanisms, most likely a combination of erasure and new learning (Quirk et al. 2010).

8.2.2 Neurobiological Models of Conditioning and Extinction

The neurobiological mechanisms by which fear is acquired have been extensively studied in the rodent. Such research has revealed that following the processing of sensory information about the CS and the US by the thalamus, the basolateral

nucleus of the amygdala (BLA) converges this information to produce a specific representation of the CS–US association (LeDoux 2007; Orsini and Maren 2012). The expression of fear responses depends on BLA activation of the central nucleus of the amygdala (CEA), which in turn activates downstream structures involved in species-specific defensive responses (e.g., the periaqueductal gray). Disruption of BLA functioning through lesions, inactivation, or administration of drug antagonists have all been shown to cause specific impairments in fear conditioning (see review by Maren and Quirk 2004). Although originally thought to be primarily involved in the expression of fear, more recent evidence has suggested that the CEA is also involved in the acquisition of fear memories, as functional inactivation of the CEA prior to fear conditioning disrupts the formation of such memories (Ciocchi et al. 2010; Wilensky et al. 2006).

Contextual fear conditioning, like cued conditioning, also depends on the amygdala (Goosens and Maren 2001). In addition, the hippocampus appears to have a specific role in contextual, but not cued, fear conditioning. Lesions to the dorsal hippocampus immediately after cued conditioning spared memory for the cue, but impaired memory for the context in which the cued conditioning took place (Anagnostaras et al. 1999). This suggests that the hippocampus is necessary for conditioning to diffuse, but not discrete, stimuli. On the basis of these and other findings, it has been suggested that the hippocampus is responsible for integrating the various sensory information about the context into one unified representation, which is then converged with the US representation in the BLA (Matus-Amat et al. 2004).

There is also evidence that the prelimbic (PL) division of the ventromedial prefrontal cortex (vmPFC) regulates amygdala activation during recall of fear conditioning. Expression of both contextual and cued fear conditioning is disrupted following PL inactivation (Laurent and Westbrook 2009; Sierra-Mercado et al. 2011), and microstimulation of PL increases conditioned fear responses and hinders extinction (Corcoran and Quirk 2007; Vidal-Gonzalez et al. 2006). In addition, freezing responses to a conditioned tone during conditioning and extinction training are positively correlated with tone responses in the PL, and persistent tone responses in the PL during recall are associated with failure to extinguish conditioned freezing (Burglos-Robles et al. 2009). Finally, disrupted consolidation of cued fear has also been reported to occur in mice with virally mediated brain-derived neurotrophic factor (BDNF) gene deletion in the PL. Viral-infected mice exhibit normal acquisition and expression of fear during conditioning, but impaired recall when tested one day later, suggesting that in addition to regulating the expression of learned fear, BDNF activity in the PL may also mediate its consolidation (Choi et al. 2010).

Rodent studies have also established that similar to fear conditioning, fear extinction involves interactions between the vmPFC and limbic structures. Specifically, it is purported that during extinction consolidation and recall the infralimbic (IL) region of the vmPFC inhibits conditioned responding by activating the inhibitory interneurons of the BLA, which in turn prevent activation of the output neurons of the CEA, thus preventing downstream activation of specific

fear responses (see Quirk and Mueller 2008, for an extensive review). Again, the hippocampus is thought to be involved in the contextual regulation of extinction memories, activating the IL only when the extinguished CS is presented in the extinction context (Corcoran and Maren 2001). The involvement of the dorsal hippocampus (DH) in the expression of extinction is supported by studies showing that temporary inactivation of the DH prior to retrieval test eliminates the renewal effect (Corcoran and Maren 2001, 2004). The DH may also be involved in the acquisition and retention of extinction, as inactivation of the DH prior to extinction training slows the rate of extinction and leads to reduced recall the following day (Corcoran et al. 2005). Finally, more recent evidence implicates the ventral hippocampus in the acquisition of extinction, as inactivation prior to extinction training, but not immediately after, causes deficits in recall the following day (Sierra-Mercado et al. 2011).

Studies using neuroimaging tools in humans have demonstrated remarkable preservation of the neural circuitry regulating both conditioning and extinction across species. The human amygdala increases activity during acquisition and recall of fear conditioning (Knight et al. 2004; Phelps et al. 2004), and decreases activity across extinction training (La Bar et al. 1998). The dorsal anterior cingulate (dAC) has also been shown to increase activity during acquisition and recall of conditioning, and there is some evidence to suggest that the thickness of the dAC cortex is correlated with fear conditioning strength (Milad et al. 2007, b; but see Hartley et al. 2011). This may suggest that the human dAC is functionally analogous to the rodent PL.

The human vmPFC has also been shown to play a specific role in extinction; hence, it may be viewed as functionally analogous to the rodent IL. vmPFC activity has been shown to increase over the course of extinction training (Gottfried and Dolan 2004). Studies examining the neurocircuitry involved in long-term recall of extinction memories have shown that vmPFC activity and thickness are both correlated with levels of extinction recall (Milad et al. 2005; 2007, b). Finally, just as in the rodent, the human hippocampus also appears to be involved in the contextual gating of extinction memories. Hippocampal activity increases during extinction recall (Knight et al. 2004; Milad et al. 2007, b), and one study has shown that hippocampus activity increases only when the CS is presented in the extinction context, and no changes in hippocampal activity occur when the CS is presented outside of the extinction context (Kalisch et al. 2006), supporting the notion that the hippocampus gates when and where extinction memories are expressed on the basis of contextual cues.

It was noted above that more recent theories of extinction postulate partial erasure of the fear memory. Research in rodents has supported at least two neurobiological mechanisms by which this could occur. The first is depotentiation, which refers to a reversal of the long-term, synaptic changes associated with long-term memory. Lin et al. (2003) demonstrated that low-frequency stimulation to the amygdala of adult rodents applied after fear conditioning induced depotentiation and reduced conditioned fear expression (i.e., caused “extinction” of fear). Kim et al. (2007) subsequently demonstrated that fear extinction caused depotentiation

of auditory fear conditioning-induced synaptic changes at thalamic input synapses onto the lateral amygdala. Second, it has recently been demonstrated that fear conditioning causes elimination of dendritic spines in the frontal cortex of mice, and that extinction causes spine formation in the same location of the eliminated spines, suggesting that extinction reverses the changes in dendritic remodeling induced by conditioning. Erasure of fear memory has also been reported to occur in humans if the inter-trial interval between the first and second CS presentations during extinction is extended; however, the neural correlates of this finding are yet to be identified (Schiller et al. 2010).

8.3 Do Fear Conditioning and Extinction Constitute Behavioral Phenotypes or Endophenotypes?

Part of the attraction to research on fear conditioning and extinction is that, as noted in the first section of this chapter, these procedures model the symptoms of anxiety along with the reductions in anxiety observed following successful treatment. The advantage of having robust laboratory models of psychiatric disorders is that they foster the development of novel treatments that can be easily tested in a preclinical context (Graham et al. 2011). However, there are concerns that laboratory models of clinical phenotypes that do not reflect the etiology of psychiatric disorders may potentially stunt progress in determining the genetic basis for such disorders (Hettema et al. 2003). In the following section, we review existing evidence examining whether fear conditioning and extinction processes extend beyond mere models of anxiety/treatment to also represent the underlying etiology and mechanisms of dysfunction in pathological anxiety. Specifically, we will examine whether certain conditioning and extinction profiles may be behavioral phenotypes or EPs that represent the genetic basis for anxiety, according to the criteria for EPs delineated by Gottesman and Gould (2003).

8.3.1 Fear Conditioning and Extinction as Behavioral Phenotypes or Endophenotypes: Evidence for Reliability

At a very basic level, a useful behavioral phenotype must be reliably measured and relatively stable. As noted previously, most studies examining fear conditioning and extinction processes in humans use psychophysiological measures of fear responses, such as potentiated startle or SCRs. These measures have the advantage of eliminating concerns about inter-rater reliability, and also circumvent the subjectivity associated with self-report regarding participants' knowledge of the CS–US contingencies (particularly as controversy exists as to whether or not

explicit awareness of such contingencies is necessary for conditioning; Lovibond and Shanks 2002). Accepting that conditioned fear responses can be reliably and objectively measured using psychophysiology, is there any evidence that conditioning and extinction abilities are stable traits? Animal research exploiting the observation of large individual differences in conditioning and extinction abilities in rodents supports the notion that specific phenotypes reflecting conditioning and/or extinction ability can be identified, and that these phenotypes are stable across testing sessions. For example, using the measure of conditioned freezing, Bush et al. (2007) separated Sprague-Dawley rats into high and low reactivity, or fast and slow recovery phenotypes, according to freezing levels exhibited during fear conditioning and extinction training, respectively. They reported that these phenotypes were consistent across subsequent tests that took place in both the conditioning and extinction contexts. Moreover, the "recovery" phenotype persisted at the follow-up time points despite both groups exhibiting comparable extinction learning by the end of extinction training. This suggests the presence of two distinct, relatively stable behavioral phenotypes in rats with respect to conditioning and extinction.

To the best of our knowledge, only one study has examined the test–retest reliability of psychophysiological indices of conditioning and extinction across time in humans. We examined conditioning and extinction ability in a population of healthy adults across three test sessions, each separated by an interval of 8–12 weeks (Zeidan et al. 2011). SCRs were used as a measure of conditioned responses. No significant differences in average fear acquisition, extinction learning, or extinction recall were found across the three time points, and responses during these phases were correlated within subjects across the three time points. This suggests that conditioning and extinction abilities can be reliably measured using SCRs and that, at least in the healthy adult population, these abilities remain stable across a course of around 24 weeks.

8.3.2 Fear Conditioning and Extinction as Behavioral Phenotypes or Endophenotypes: Evidence for Heritability

In addition to being reliable, Gottesman and Gould (2003) stipulate that behavioral phenotypes should be heritable. Animal studies have provided some evidence to suggest that fear conditioning and extinction abilities are heritable traits. Such studies have reported the existence of strain differences in conditioning and/or extinction profiles, suggesting that these phenotypes can be selectively bred. For example, Hefner et al. (2008) reported significant differences in extinction recall between two inbreeds of mice, despite there being no differences in fear acquisition or extinction learning. Moreover, extinction in the impaired breed was associated with reduced activity in the IL and BLA, and was unresponsive to treatments

that normally enhance extinction recall (e.g., increased extinction training trials or pharmacological adjuncts). Similar findings have been reported for Wistar rats selectively bred for high- and low-anxiety-related behavior (Muigg et al. 2008). Despite showing comparable fear acquisition to low-anxiety rats, high-anxiety rats exhibited impaired extinction learning and recall, and reduced activity in IL and lateral amygdala.

The few studies that have examined heritability of fear conditioning and extinction in humans have revealed similar results to those reported in rodents. For example, Hettema et al. (2003) examined fear conditioning and extinction learning in a population of healthy monozygotic and dizygotic twins. There were higher correlations in conditioning, and extinction rates between monozygotic than dizygotic twins, and the authors reported that genetic heritability accounted for 35–45% of the variance associated with these rates. Thus, this study supports the idea of conditioning and extinction being moderately heritable traits in humans. Furthermore, this study also reported evidence suggesting that heritability of conditioning and extinction to ecologically relevant fear stimuli (e.g., snakes and spiders) may be greater than that to neutral fear stimuli (e.g., shapes). Given that many phobias occur to ecologically relevant stimuli and that humans preferentially condition to stimuli that were ecologically relevant to the pre-technical man (Mineka and Öhman 2002), this might suggest that the use of such stimuli in laboratory tasks may be optimally suited to detect genetic substrates of conditioning/extinction processes that are relevant to the etiology of anxiety. The findings from Hettema et al. (2003) also fit with previous reports that correlations between eyeblink conditioning rates are higher in monozygotic than dizygotic twins (Merrill et al. 1999). Although eyeblink conditioning is not strictly considered "fear" conditioning, it is mediated by an associative learning process. Together, these studies do support the notion that associative learning, the theorized cognitive mechanism underlying conditioning and extinction, is at least somewhat heritable.

8.3.3 Fear Conditioning and Extinction as Behavioral Phenotypes or Endophenotypes: Association with Anxiety Disorders

In order to be considered as behavioral phenotypes for anxiety disorders, specific conditioning and extinction phenotypes should be implicated in the etiology of anxiety. Earlier behavioral/learning accounts of anxiety disorders were subject to the criticism that they failed to account for the complexity of individual differences regarding the psychological ramifications of traumatic events. That is, not everyone who experiences a conditioning episode (i.e., a trauma) develops anxiety, and not everyone with an anxiety disorder can recall a specific conditioning episode that precipitated the disorder (Rachman 1990). In the last two decades, however, more contemporary learning models of anxiety have been developed that

consider factors such as conditioning through vicarious rather than directly experiential means, the nature of the event (i.e., controllable versus uncontrollable), and the impact of pre- and post-event variables (such as learning history), to better account for the complexity of individual differences in the development of anxiety. As a result, the view is now well accepted that learning processes underlying fear conditioning and extinction, combined with temperamental/personality vulnerabilities, can at least partly account for the development and maintenance of anxiety disorders. An extensive review on the evidence supporting this account is beyond the scope of this chapter; however, the interested reader should refer to Mineka and Zinbarg (2006) for an excellent review on this topic.

Accepting the relevance of conditioning and extinction processes in the etiology and course of anxiety, it next needs to be determined whether people with clinical anxiety exhibit specific conditioning and extinction phenotypes. Indeed, there is much evidence to suggest that clinical anxiety is associated with heightened conditionability and/or impaired extinction. For example, a recent meta-analysis that reviewed 20 studies of laboratory conditioning and extinction tasks in a range of anxiety disorders demonstrated moderately enhanced conditioned responding during conditioning and extinction in people with anxiety disorders relative to healthy controls (Lissek et al. 2005). This analysis mainly included studies that required participants to learn about and subsequently extinguish fear to simple, single cues. Other studies comparing responses to a conditioned cue versus a "safety" cue (i.e., a cue that was never reinforced) have revealed that people with post-traumatic stress disorder (PTSD) tend to exhibit higher levels of conditioned responding to both the conditioned cue as well as the non-reinforced cue, suggesting a diminished ability among people with PTSD to discriminate between dangerous and safe cues (Blechert et al. 2007; Norrholm et al. 2011; Orr et al. 2000; Peri et al. 2000). These latter studies also reported delays in subsequent extinction, which may merely be a reflection of heightened acquisition during conditioning, or may reflect an additional impairment in fear extinction.

It does appear that anxiety is also associated with deficient extinction, beyond its association with initial conditioning strength. Recent studies have reported specific failures in extinction learning or extinction recall, despite there being no differences in fear conditioning, in anxious populations. This has been demonstrated in people with panic disorder using both SCRs and valence ratings as indices of conditioned fear (Michael et al. 2007). We have reported that people with PTSD exhibit impairments extinction recall, despite there being no differences in conditioning or extinction learning (Milad et al. 2008, 2009).

PTSD impairment in safety learning has also been reported in a different model of fear inhibition that examines the ability to suppress fear responses when a CS is shown in the presence of a conditioned inhibitor (i.e., a safety signal). Compared to healthy controls, PTSD participants exhibited reduced suppression of potentiated startle in trials that included the conditioned inhibitor (Jovanovich et al. 2009). This finding was recently replicated in different cohorts of participants with PTSD, and moreover, the impairment in conditioned inhibition was not detected in participants with major depressive disorder (Jovanovic et al. 2010). This suggests

that impaired fear inhibition may be specific to anxiety disorders, rather than a reflection of psychiatric distress in general.

The alterations in conditioning and extinction appear to be related to symptom severity, where the greater the severity the more heightened the conditioning, and/or the more impaired the extinction ability (Milad et al. 2009; Norrholm et al. 2011). In addition, these alterations are associated with differences in the neural circuitry underlying fear conditioning and extinction. For example, using positron emission tomography, Bremner et al. (2005) demonstrated that people with PTSD exhibited heightened behavioral responses during fear acquisition and extinction that were associated with increased resting metabolic activity in the left amygdala, and decreased resting metabolic activity in the ventromedial prefrontal cortex, respectively, compared to healthy controls. We reported that the impaired extinction recall observed in PTSD populations is associated with reduced activity in the vmPFC and hippocampus, but heightened dAC activity to conditioned cues (Milad et al. 2009) and contexts (Rougemont-Bücking et al. 2011). This suggests that behavioral or psychophysiological measures of conditioning and extinction ability in anxious populations may tap into underlying dysfunctions in cortical and limbic regions that mediate emotion regulation.

8.3.4 Fear Conditioning and Extinction as Behavioral Phenotypes or Endophenotypes: Issues of Co-segregation and State-Independency

The previous section described evidence that dysfunctions in acquisition and extinction of fear are associated with anxiety disorders, and that these dysfunctions are captured in a variety of laboratory tasks across different anxiety subtypes. The question remains, however, whether these dysfunctions represent genetic vulnerabilities to the development of anxiety, or whether they are merely epiphenomenal to the general pathology. One way to assess this is to determine whether fear conditioning/extinction phenotypes and anxiety disorders "co-segregate" in family members (Gottesman and Gould 2003). The first study to examine this compared the genetic covariation between psychophysiological measures of conditioning/extinction profiles and self-reported phobic fears in monozygotic and dizygotic twins (Hettema et al. 2008). A surprising negative correlation was found between psychophysiological fear responses and self-reported phobic fears, and genetic factors underlying fear conditioning/extinction accounted for only 9% of individual differences in self-reported phobic fears. The authors suggested that their data should caution against the use of fear conditioning as a behavioral phenotype for specific phobia.

Likewise, in our examination of a population of monozygotic twins discordant for trauma exposure and PTSD, we observed that extinction recall was impaired in PTSD participants but not their non-trauma exposed co-twin, relative to non-PTSD twins discordant for trauma exposure (Milad et al. 2008). Of course, both

our and Hettema et al.'s (2008) findings do not preclude the possibility of a gene-by-environment interaction, whereby the non-affected co-twin may be genetically vulnerable, but that this vulnerability will only manifest after exposure to trauma. These studies do suggest that impaired extinction may not be a reflection of a pre-existing genetic factor, and further, that it is not a consequence of trauma exposure per se. Rather, impaired extinction may be a specific consequence of the development of PTSD.

Gottesman and Gould (2003) have also stipulated that a psychiatric behavioral phenotype should be present in an individual regardless of whether or not the illness is active (i.e., it should be state-independent). As successful treatment of anxiety may eventually alter the conditioning/extinction behavioral phenotype (even if it is the underlying cause of the disorder), a prudent way to assess this criterion would be to examine whether the phenotype exists *prior to* symptom development, and thus may be predictive of the eventual development of anxiety. Such prospective studies are difficult to conduct; however, Guthrie and Bryant (2006) examined fear conditioning and extinction learning in firefighters during cadet training using SCRs and corrugator electromyography (EMG) responses as indices of conditioned responses. Participants were reassessed for PTSD 24 months post-training following trauma exposure. Heightened EMG responses during extinction training at the time of cadet training accounted for 31% of the variance associated with subsequent PTSD symptomatology two years post-cadet training. This study suggests that impairments in extinction may be moderately predictive of vulnerability to anxiety and challenges the previously described studies suggesting that impaired extinction is merely a consequence of anxiety.

8.4 Conclusion

Considerable research over the past decades has explored the behavioral, cognitive, and neurobiological mechanisms underlying conditioning and extinction in rodents, and more recently, in humans. Evidence suggests that conditioning and extinction abilities are altered in clinically anxious populations, and that these alterations are reflected by changes in the neural circuitry that mediates such abilities (Milad et al. 2009; Rougemont-Bücking et al. 2011). In addition, it is accepted that learning processes underlying conditioning and extinction at least partly mediate the development and maintenance of anxiety disorders. Despite this, there is a dearth of research that has examined whether the conditioning and extinction profiles observed in anxiety are genetically acquired. Thus, it is difficult to draw firm conclusions as to whether current models of conditioning and extinction measure behavioral phenotypes that reflect the genetic factors underlying anxiety. The few studies that have examined whether deficits in fear extinction associated with anxiety are also seen in first degree; unaffected relatives have indicated that the deficits are specific to those inflicted with the disorder (Hettema et al. 2008; Milad et al. 2008). The one study that has examined fear extinction ability as a

predictor of future anxiety has revealed that impairments in extinction can account for a considerable amount of the variance associated with subsequent PTSD symptoms (Guthrie and Bryant 2006). There are at least two explanations for these apparently inconsistent findings: First, it is possible that heightened conditioning/impaired extinction profiles are consequences of anxiety disorders, and thus do not constitute true behavioral phenotypes. This explanation would account for the lack of co-segregation of conditioning/extinction profiles and anxiety disorders within monozygotic twins (Hettema et al. 2008; Milad et al. 2008). This explanation would also be consistent with the postulated role for conditioning/extinction processes in the *maintenance* of anxiety disorders, in the sense that once an anxiety disorder is acquired, the consequent impaired extinction ability would serve to prevent natural extinction of the anxiety and potentially impede the impact of exposure-based treatments. However, this explanation does not account for the finding that extinction impairments precede PTSD symptom onset (Guthrie and Bryant 2006). Moreover, it is inconsistent with evidence that learning processes prior to, during, and subsequent to traumatic events contribute to the initial development of anxiety (Mineka and Zinbarg 2006).

A second possible explanation is that specific conditioning/extinction profiles are predisposing vulnerabilities to anxiety, but that these vulnerabilities are acquired (e.g., through early-life experiences) rather than genetic in origin. This explanation would account for Guthrie and Bryant's (2006) report of pre-existing deficiencies in extinction in people who develop PTSD symptoms, but would also account for the apparent lack of such deficiencies in non-affected monozygotic co-twins (Hettema e al. 2008; Milad et al. 2008). On the face of it, this explanation may appear to be contrary to reports that conditioning/extinction phenotypes are heritable (Hettema et al. 2003). However, when it is considered that all phenotypes will represent a combination of genetic and environmental factors, it is feasible to consider the proposition that in some cases, environmental experience may overshadow the impact of genetics on conditioning/extinction profiles, hence leading to null effects in co-segregation studies. Indeed, the idea that conditioning/extinction profiles can be modified by life events is supported by preclinical studies in rodents that have demonstrated that early-life maternal deprivation (Callaghan and Richardson 2011, 2012), early-life exposure to neurotrophic factor (Graham and Richardson 2010), or chronic stress (Izquierdo et al. 2006; Miracle et al. 2006) all impact conditioning and/or extinction abilities later in life.

Another potential reason for the apparently discrepant findings regarding whether conditioning/extinction traits are acquired versus pre-existing may be that some studies have focused on conditioning and extinction learning, and others have focused on longer-term retention of the extinction memory. Animal studies support the idea that the three phases of the model (conditioning, extinction learning, and extinction recall) may be distinct phenotypes controlled by discrete neurocircuitry. It is possible that not all of these subphases are equally relevant to/informative about the origin and maintenance of anxiety disorders. In fact, a recent review of exposure processes in clinical anxiety has demonstrated that there is little evidence for correlations between initial fear response or within-session

extinction (i.e., extinction learning) and between-session extinction, referring to the maintenance of the extinction memory across repeated sessions (Craske et al. 2008). This notion has also been supported by preclinical studies in rodents (Plendl and Wotjak 2010). Given that preserved between-session extinction is clearly necessary to maintain treatment gains over the longer term, it may be the case that deficient extinction recall is the more relevant phenotype of anxiety rather than initial conditioning strength or within-session extinction learning. It is possible that if future studies focus on the extinction recall phase, more consistent findings regarding the contribution of conditioning/extinction processes to the genetic factors underlying anxiety will emerge.

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